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Seasonal Influence on Hematology and Biochemical Blood Parameters in Mongolian Domestic Sheep

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ABSTRACT

Mongolian livestock are most exposed to cold and nutritional stress during the winter and spring. This study examines the seasonal variations in hematological and biochemical parameters, including cortisol hormone levels, in Mongolian domestic sheep. The results showed an increase in white blood cell count and a decrease in red blood cell count and hematocrit during winter and spring. Additionally, there were decreases in serum total protein, albumin, triglyceride, and calcium, along with significant increases in high-density lipoprotein, urea and cortisol concentrations in winter and spring compared to autumn. These fluctuations maintained a stable level of homeostasis, indicating that Mongolian domestic sheep possess a high biological ability to adapt to adverse influences such as ecosystem, climate, and pasture shortage.

Key words: Sheep, Hematology, Biochemistry, Cortisol, Stress

INTRODUCTION

Animal husbandry is a key foundation of the Mongolian economy, with traditional grazing livestock that relies on the ecosystem and climate playing an essential role in this sector. Sheep and goats account for 85 percent of Mongolia's five livestock species (Mongolian Statistical Information Service 2024). Mongolian grazing livestock have developed unique biological and physiological adaptations due to their survival in a harsh climate with four distinct seasons and minimal human interaction. In other words, Mongolian animals live and thrive in a rhythm that depends on the seasonality of their body systems and metabolism. During the summer and autumn seasons, which are the most favorable in terms of climate and nutrition, the body of Mongolian livestock is dominated by assimilation to replenish and store energy materials. In the winter and spring seasons, adverse climatic conditions and food shortages often coincide, leading to defensive responses dominated by dissimilation to maintain homeostasis. While winter and spring are natural seasons for grassland ecosystems, recent years have seen an increase in the frequency of catastrophic weather events due to global warming. For example, the winter of 2024 was colder than average for many years (Mongolian Statistical Information Service 2024) and heavy snowfall led to "zud" conditions, resulting in the death of more than 7 million livestock due to the effects of the cold and malnutrition.

Factors that cause stress in pastures primarily include adverse weather conditions, such as extreme heat and cold, as well as malnutrition and starvation, among others. Beyond causing physiological changes, these stressors can lead to significant dysfunction and behavioral changes in animals and in severe cases, even death (Kumar et al. 2012). Cold stress can be activated by the sympathetic nervous system in the body, which activates the sympathetic adrenal system and increases the amount of cortisol in the blood (Doubek et al. 2003; Debnath et al. 2024). This rise in cortisol enhances the utilization of energy compounds within cells, leading to an increased breakdown of complex compounds and heightened energy production (Doubek et al. 2003). Consequently, cortisol concentration in the blood serves as one of the main indicators of the body's stress levels (Kim et al. 2023; Wang et al. 2023; Alomar and Zarkawi 2024).

Also, blood cells and serum metabolic parameters are likely to change depending on environmental conditions, climate, and nutritional quality. For example a pattern of decreased red blood cell (RBC) counts and increased white

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blood cell (WBC) counts in winter has been observed in the Awassi sheep of meat and dairy sheep, which are widespread in Central Asia and Europe (Alomar and Zarkawi 2024) and RBC counts and hemoglobin (HB) concentrations have also been found to decrease in Indian sheep during the winter (Rathwa et al. 2016). Also, in the warmer region of Sudan, RBC counts and hematocrit (HCT) levels in Nubian goats were found to be highest in humid summer and lowest in dry summer and winter (Abdelatif et al. 2009).

Many studies have reported that some serum biochemical parameters such as total protein (TP), albumin (ALB), glucose (GLU), urea (BUN), cholesterol (CHOL), triglyceride (TG), etc. changed by the effect of cold stress and malnutrition. A protein breakdown in tissue can increase due to the effect of stress factors including cold, heat, malnutrition, transportation, etc. Awassi sheep have been shown a low concentration of TP and a high concentration of BUN during the quality of pasture and cold stress (Al-Musawi et al. 2017). Similarly, minimum and low concentrations of TP and ALB have been measured in Sudanese Nubian goats (Abdelatif et al. 2009), Tyvan short-fat-tailed sheep (Shimit et al. 2020) and "Holstein Heifers" cattle (Rasooli et al. 2004) in winter. In addition, the concentration of CHOL and HDL has been increased in cattle (Holstein Heifers), pregnant ewes (Württemberge), and Mongolian sheep in the Southern Mongolian Plateau, Inner Mongolia during the winter season (Doubek et al. 2003; Rasooli et al. 2004; Zhang et al. 2022). A comparative study of serum biochemical parameters in Mongolian sheep adapted to the Southern Mongolian Plateau, Inner Mongolia in winter reported that glucose and fat metabolism was promoted and HDL concentrations increased (Zhang et al. 2022). Also, low concentration of TG has been observed in cattle (Simmental crossbred), Bulgarian localized sheep (Lacaune), and lamb (Württemberger) exposed to both short- and long-term cold stress (Doubek et al. 2003; Nedeva et al. 2022; Wang et al. 2023).

In our country, pasture grass reserves are reduced to a minimum in winter and spring, causing livestock to suffer from nutritional deficiencies and cold stress. This study aimed to compare the seasonal changes in hematology, biochemistry, and some hormonal parameters of Mongolian sheep in the steppe region during autumn, winter, and spring.

MATERIALS AND METHODS

The study was conducted in Tuv province, a steppe region of central Mongolia and involved clinically healthy, non-pregnant ewes aged 2-4 years, which were kept in a traditional husbandry setting. Clinical examination included evaluation of temperature, skin, mucous membranes, cardiopulmonary, gastrointestinal and neurological systems. The study was conducted in September 2023 (autumn month), January 2024 (winter month), and March 2024 (spring month) and 40 selected sheep were sampled in each sampling time.

All samples were collected from jugular vein blood after clinical examination. Hematological parameters such as hematocrit by a HematoSpin centrifuge, and red blood cell count (RBC) and white blood cell count (WBC) were estimated by cell counting methods using a hemocytometer. Whole blood for biochemical and hormone examination was collected in a 7mL tube using serum separator tubes according to standard procedures, and the serum samples were stored at -20℃ until analysis. Biochemical blood indicators included serum total protein (TP), albumin (ALB), alkaline phosphatase (ALP), glucose (GLU), total bilirubin (TBIL), total cholesterol (T.CHOL), gamma-glutamyl transferase (GGT), glutamic oxalacetic transaminase (GOT), glutamic-pyruvate transaminase (GPT), urea (BUN), amylase (AMYL), lipase (v-LIP), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), creatinine (CRE), calcium (Ca), inorganic phosphate (IP), and electrolytes (Na-K-Cl) which were determined by an automatic biochemistry analyzer (Dri-Chem NX500i, Fujifilm, Japan) according to the instrument's instructions. Estimation of cortisol concentration in serum was performed using commercial enzyme-linked immunosorbent assay (ELISA) kits (Enzo Life Sciences, Inc.,) according to the manufacturer's instructions. The ELISA results were obtained using a microplate reader (Multiskan EX, Thermo Scientific).

RESULTS & DISCUSSION

During the sampling period, mean temperatures were coldest in winter at -17.8°C and warmest in autumn at 4.1°C, and precipitation was highest in winter, with 20–25cm of snowfall in the pastures (Table 1). The National Agency of Meteorology and Environmental Monitoring determined that precipitation for the year was 99.7 percent higher than the long-term average (The National Agency Meteorology and the Environmental Monitoring 2024). Environmental factors such as temperature (heat, cold), relative humidity and solar radiation can cause discomfort and stress to animals, greatly affecting animal physiology (Sejian et al. 2018). The neutral temperature range for sheep is generally reported to be between 12 and 27°C (De et al. 2017). However, different species have different thermal comfort zone temperature values for optimal production and the thermal sensitivity of species changes with age and physiological stage (Tüfekci and Sejian 2023).

Table 1: The temperatures and precipitation during the sampling period

Variables	Autumn	Winter	Spring
Average temperature (ºC)	4.1	-17.8	-7.6
Multi-year average precipitation	Near	99.7% higher*	near

**Snow*

Blood is the vehicle for transporting nutrients and metabolic products throughout the body and is the pathway by which fluid regulation is achieved. Therefore, when the body is exposed to any stress, biochemical parameters change to ensure energy metabolism. Theoretically, the hematological studies are of ecological and physiological interest in understanding the relationship between blood characteristics and the environment (Ovuru and Ekweozor 2004). Domestic animals fed natural pasture is a major factor that can negatively affect animal performance. In Mongolia, there is a significant shortage of forage plants in pastures from winter to spring and depending on the thickness of the snow cover, grazing livestock can suffer from a lack of forage.

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The hematological and biochemical parameters of Mongolian domestic sheep changed from autumn to winter and spring due to extreme weather conditions and nutritional deficiencies (Table 2). However, most parameters are within normal reference ranges allowing homeostasis to be maintained. For example; there were no significant changes in some biochemical parameters including ALP, GLU, TBIL, T.CHOL, GOT, GPT, GGT, AMYL, v-LIP, CRE, IP and Na-K-Cl.

Table 2: The seasonal variations on hematological and biochemical parameters of Mongolian sheep

Parameters	Season		
	Autumn	Winter	Spring
RBC (10^3/μL)	11.84±0.26	7.45±0.15	5.68±0.10
HCT (%)	38.9±0.89	28.7±0.9	25.5±0.82
WBC (10^3/μL)	5.77±0.13	6.88±0.12	8.35±0.15
TP (g/dL)	7.24±0.03	6.02±0.12	6.35±0.04
ALB (g/dL)	3.82±0.02	2.45 ±0.06	2.47±0.03
BUN (mg/dL)	11.49±0.58	16.53±0.63	18.80±0.28
TG (mg/dL)	13.70±0.72	5.17±0.46	5.06±0.96
HDL (mg/dL)	30.2±0.781	180.19±1.08	453.73±1.12
Ca (mg/dL)	12.11±0.68	9.33±0.72	8.89±0.38

It has been shown that when sympathetic nervous activity is increased due to cold stress in winter, the number of WBC increases, and the breakdown of adipose tissue increases HDL concentration and decreases TG concentration (Table 2). Generally, cold stress improved energy expenditure in ruminants, leading to increased use of lipids as an energy substrate. Similar to our finding, Rasooli et al. (2004) and Zhang et al. (2022) reported the increased concentration of HDL in cattle and sheep during the winter season, respectively. Also, several studies observed a decrease in TG concentrations in cattle, sheep, and lambs when exposed to short-term and long-term cold stress (Doubek et al. 2003; Hu et al. 2021; Nedeva et al. 2022; Wang et al. 2023).

Additionally, it was observed that the levels of RBC, HCT, TP, ALB, and Ca significantly decreased from autumn to spring (Table 2). This decline indicates that the nutritional deficiencies that began in winter have continued into spring, and the breakdown of adipose tissue is continuing. One of the major challenges in livestock husbandry is seasonality and nutritional status (Alam et al. 2011), especially in Mongolia. Similar studies reported decreases in RBC and HB concentrations in Awassi and Indian local sheep during winter (Rathwa et al. 2016; Alomar and Zarkawi 2024). Furthermore, other studies have shown that low concentrations of TP and ALB in sheep, goats, and cattle are often linked to cold stress and low nutritional quality (Rasooli et al. 2004; Abdelatif et al. 2009; Al-Musawi et al. 2017; Shimit et al. 2020). Low total serum protein in an animal, also known as hypoproteinemia, can indicate a variety of underlying problems, including nutritional deficiencies, liver or kidney disease, or protein-losing states. This phenomenon may also be explained by the fact that cold stress triggers a complex series of cellular responses ranging from the inhibition of general protein synthesis to the activation of specific cold-responsive genes and proteins (Debnath et al. 2024).

As a result, the concentration of BUN elevated steadily in the winter and spring compared to the autumn (Table 2). This result is consistent with other studies, such as Al-Musawi et al. (2017) which reported that poor pasture quality and cold stress led to increased BUN concentrations. BUN can be also increased by numerous extrarenal factors including prolonged fasting (starvation), because of the catabolism of body proteins. Urea is the waste product of protein catabolism.

Besides, results demonstrated an increase in serum cortisol concentration in the winter and spring season compared to autumn in Mongolian domestic sheep (Table 3). These results indicate that Mongolian domestic animals are exposed to cold and nutritional stress during the winter and spring seasons. Because glucocorticoid hormones play an important role in regulating metabolism, immune responses, stress responses and overall adaptation to exposure to environmental, physiological, nutritional, and managemental stressors (Sahib et al. 2024). Many studies reported that environmental stress such as cold stress can be elevated serum cortisol concentration in animals (Kim et al. 2023; Wang et al. 2023; Alomar and Zarkawi 2024). Also, nutritional stressors such as starvation, malnutrition, low quality of feed and pasture can increase blood cortisol concentration (Bajaj et al. 1979; Gort-Esteve et al. 2024). For example; Bajaj et al. (1979) reported that plasma cortisol levels are elevated in states of protein malnutrition. Similarly, some study reported that increase in glucocorticoid hormone levels during prolonged starvation (Kitaysky et al. 2005). Sejian et al. (2014) investigated the effects of 60% food restriction on growth, physiological adaptation and reproduction in sheep. In this case, food restriction significantly decreased body weight and body condition score and significantly increased plasma cortisol. Cortisol is the main stress-relieving hormone and triggers a variety of physiological changes in these sheep to adapt to the harsh temperature conditions (Sejian et al. 2017).

Table 3: The seasonal variations in serum cortisol hormone of Mongolian sheep

Parameter	Season		
	Autumn	Winter	Spring
Cortisol (ng/mL)	4.28±3.79	17.48±5.63	20.36±5.23

Conclusion

Mongolian sheep on pasture have the biological characteristic of accumulating adipose tissue to survive the severe cold season from summer to autumn when the natural climate and nutrition are most favorable.

In winter, the cold weather increases the amount of cortisol in the blood, which activates the breakdown of fat and steadily increases the amount of HDL in the serum. As the physiological processes aimed at overcoming the effects of cold and nutrient deficiency continue for a long time from winter to spring, the breakdown of not only fats but also proteins occur, protein synthesis decreases, and the levels of total serum protein and albumin fall to low levels. As the body breaks down protein, the amount of urea in the serum increases. Following this pattern, red blood cell count, hematocrit, and serum macronutrient levels steadily decline starting in winter.

However, these fluctuations are at a homeostatic level, indicating that Mongolian domestic sheep have a high biological capacity to adapt to adverse ecosystem, climatic, and pasture scarcity conditions.

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DECLARATIONS

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Data Availability: The data presented in this study are available on request from the corresponding author.

Ethics Statement: The animal studies were approved by Animal Care and Use Committee, Institute of Veterinary Medicine, Mongolian University of Life Sciences (Agreement Number № MEBUS-23/10/09). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

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